

REMARKS**I. Detailed Action*****A. Continued Examination Under 37 C.F.R. § 1.114***

Applicants acknowledge that the Examiner states that "this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114."

B. Drawings

Applicants acknowledge that the Examiner has acknowledged the Applicant's petition to accept color photographs filed under 37 C.F.R. § 1.84(a)(2).

C. Claim Objections

Claim 10 is objected to because the claim ends in two periods. Applicants have amended the claim, thereby alleviating the rejection. Applicants thank the Examiner for pointing out this inadvertent mistake.

D. New Claim

Applicants have added new dependent claims 40 and 41 and ask the Examiner to consider it for allowance. No new matter has been added (see specification, p. 8).

II. Claim Rejections***A. 35 U.S.C. § 112, First Paragraph***

Claim 4 was rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to

which it pertains, or with which it is most nearly connected, to make and/or use the claimed invention.

The Applicants have deleted claim 4, thereby alleviating this rejection.

Claims 1-3, 9-14, 16-22, 24 and 38-39 were rejected under 35 U.S.C. § 112, first paragraph because the specification allegedly is not enabling for methods of increasing or inducing cold or freezing tolerance in plants other than wheat.

Amended claim 1 relates to a method of increasing or inducing cold or freezing tolerance in a gramineae species plant:

Wheat experiments

The Applicants acknowledge that the Examiner considered the application enabling for at least a method of increasing or inducing cold or freezing tolerance in the wheat cultivar Glenlea (page 5 of Office Action). As examples of the enabling support in the application, Figs. 1A and 1B showed that survival of plants treated with betaine during cold acclimation was dramatically improved over plants that were cold acclimated in the absence of betaine. Fig. 2 showed the results of an experiment for betaine-treated, cold acclimated plants compared to plants treated with betaine at 25°C. The results showed a dramatic improvement to the normal genotypic potential to tolerate freezing.

The application also discusses a method using wheat generally because one of skill in the art would appreciate that the claimed method would work with other wheat cultivars and wheat generally. For example, on page 6, lines 12-18, it was shown that, as in Glenlea, another wheat cultivar, Frederick, has increased betaine content during cold acclimation compared to growth at non-acclimated temperatures. In view of the surprisingly strong Glenlea results described in the application from combining cold acclimation and betaines, particularly that shown in Figures 1

and 2, the additional increase in betaine content obtained by application of exogenous betaine to Frederick during cold acclimation would be expected increase or induce freezing tolerance. One of skill in the art would readily appreciate that the methods of the invention are readily applicable and useful for other wheat varieties.

Golf turf and barley experiments

The application also shows that the claimed method is useful in increasing cold and freezing tolerance in other plant species. In particular, the application describes experimental results for golf turf and barley. The application therefore provides enabling support for claim 1 by describing experimental results that directly show that the methods of the invention work with at least three species of gramineae. These results allow one of skill in the art to conclude that the claimed methods will work, and are enabled, across the scope of the claim to methods using gramineae.

Dependent claim 16 refers to the species, wheat, golf turf and barley.

Claim 17 refers to methods using golf turf. New dependent claims 40 and 41 and refer to wheat and barley, respectively.

Successful methods of improving cold and freezing tolerance in golf turf are described on page 10, lines 17-20 and in the photographs in figures 4 and 5 and their legends. The rapid regrowth and greening in the betaine treated area reflects healthier winter survival of betaine treated turf because of increased cold and freezing tolerance.

The Applicants also determined that the combined treatment of barley with betaines and low temperature acclimation improved freezing tolerance in barley (page 8, lines 19-22). The improvement in freezing tolerance in barley was as efficient as that for wheat.

Betaines

The application describes methods using various types of betaines. Glycine betaine was described in the application as a useful betaine (eg. page 3, lines 18-20). The Applicants also describe betaine free base in water (page 4, lines 33-34). Table I on page 5 describes other betaines that are also useful in the methods of the invention.

Varied doses and dosing regimens

The application also provides multiple examples of useful doses and dosing regimens. In relation to a wheat example, it is indicated that a concentration of glycine betaine having a concentration lower than about 500mM is useful, such as 250mM (page 3, lines 18-20). Figure 1 shows an experiment with plants treated for four days at 100, 250 and 500mM glycine betaine at 6°/2°C and then cold acclimated for 30 days. Betaine may also be administered before, during and at the end of cold acclimation (page 8, lines 26-28). In another embodiment, golf turf was treated three times at a weekly interval with 1 L per square meter of 200mM betaine free base in water (page 4, lines 33-34).

Acclimation temperatures

One of skill in the art would be able to practice the invention at other acclimation temperatures. The wheat example shows cold tolerance increasing with an acclimation temperature of 6°C during the day and 2°C at the night. It is apparent to one skilled in the art that other acclimation temperatures could be used. The Applicants provide this guidance on page 3, line 2, by stating that the plant is acclimated to a temperature not lower than the coldest temperature that the plant can withstand. Information about cold-hardiness of commercially important plants should be readily available. In cases where this information is unavailable, the Applicants provide a method to determine the coldest temperature that a plant can withstand by reference to determining the LT50, the temperature at which half of the plants die. One skilled in

the art would still be able to obtain the benefit of the invention without having a precise LT50 value. For example, the application indicates on page 10, line 39 that the invention is usefully applied before a predicted frost or sudden decrease in temperature.

Improved plant physiology in cold or freezing temperatures

The application also provides detailed support for the improvement of photosynthetic capacity and overall physiology at cold temperature as well as the increase or induction of tolerance to salinity and water stress. These results are applicable to other species of gramineae. For example, the applicants show that WCOR410 is specifically induced to high levels by betaine exposure and cold acclimation and that WCOR410 is a major contributor to the improvement of freezing tolerance (page 9, lines 25-28; Fig. 4). Thus, the application provides certainty and predictability on the mechanism of action of betaines and cold acclimation in order to increase freezing tolerance. Photosynthesis is discussed on page 10, for example, lines 13-16. The Applicants have shown not only a strong result from the combination of betaines and cold acclimation, but they have provided a rational basis to explain the effects of the combination of betaines and cold acclimation in gramineae generally.

In view of the entirety of support in the application, the Applicants submit that the application provides enabling support for the currently claimed methods of inducing cold or freezing tolerance in gramineae plants.

B. 35 U.S.C. § 112, Second Paragraph

Claim 1, and its dependent claims, were rejected for allegedly omitting essential steps.

Amended claim 1 refers to acclimating said plant by "exposing said plant" to a temperature. The amended claim also refers to "administering" betaine or a derivative thereof to

said plant to increase betaine or betaine derivative concentration in said plant "during cold acclimation." It is submitted that recitation of "exposing" and "administering" provides positive method steps and overcomes the Examiner's objections.

Claims 24 and 38-39 were rejected as indefinite. The claims have been amended to delete reference to "grasses."

C. 35 U.S.C. § 102

Claims 1-3, 9, 12-14, 16, 18, 22, 24, and 38 were rejected under 35 USC § 102 as anticipated by Rajashekar et al. which was previously cited by the Examiner

Rajashekar et al. relates to applying glycine betaine to strawberry. Since the amended claim 1 refers to gramineae, it is submitted that claim 1 is not anticipated because Rajashekar et al. does not disclose all the elements of claim 1. Furthermore, Rajashekar et al. does not teach or suggest a method using gramineae that induces cold tolerance over and above that of the normal genotypic potential induced by each step alone. Rajashekar et al. also does not teach or suggest a method in gramineae that decreases the lethal temperature of a plant, that can increase freezing tolerance in wheat by at least 6°C or that results in improved regrowth, greening and resistance to photoinhibition. There is no mention of such advantages in Rajashekar et al. so one skilled in the art would have no motivation to perform the method of claim 1 with gramineae and would have no reasonable expectation of success.

D. 35 U.S.C. § 103(a)

The Examiner has rejected claims 10-11, 17, 21 and 39 as unpatentable over Rajashekar et al. in view of Kishitani and in light of Zhao (both previously cited). Kishitani relates to barley plants, and Zhao relates to alfalfa.

The Applicants acknowledge the Examiner's statement on page 11 of the Office Action that claims 19 and 20 are free of the prior art due to the failure of the prior art to teach or suggest a method of increasing or inducing cold or freezing tolerance in gramineae species plants by acclimating a plant and increasing the concentration of betaine or a derivative thereof at a concentration of about 250mM.

The Applicants also acknowledge the Examiner's statement on page 10 of the Office Action that "The Examiner acknowledges the results set forth in the disclosure for wheat, but maintains that the rejected claims are obvious because they are not limited to the exemplified method which demonstrates a significant synergistic effect when cold acclimation and betaine application are combined."

The Applicants submit that the former claim 1 wording did define by invention by stating that *combined* steps a) and b) increased the cold or freezing tolerance of said plant over and above the cold or freezing tolerance of the normal genotypic potential induced *by each step alone*. However, the Applicants have amended claim 1 to refer to "... administering betaine or a derivative thereof to said plant to increase betaine or betaine derivative concentration in said plant *during* cold acclimation..." (emphasis added). The claim continues by stating that, "...the combination of increased betaine or betaine derivative concentration in said plant *during* acclimating said plant increasing or inducing..." (emphasis added). This emphasizes that there must be some concurrency between the increased betaine concentration and acclimation. Dependent claim 9 further defines the invention by reference to the steps being substantially simultaneous.

Claim 1 therefore reflects the inventors' surprising and unexpected finding that one could increase tolerance above the known genotypic capacity to cold acclimate. There is clearly a

synergistic effect from the combination of betaines and cold acclimation. It would not be obvious to one skilled in the art that a significant synergistic increase in freezing tolerance would be obtained from the claimed method.

III. Conclusion

In light of the above amendments and remarks, Applicants respectfully assert that claims 1-4, 9-14, 16-22, 24, 38, 39 and 40 are now in condition for allowance. Applicants respectfully request reconsideration and withdrawal of the above rejections. If it is felt that it would aid in prosecution, the Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

This is a request to extend the period for filing a response in the above-identified application for two months from December 23, 2003 to February 23, 2004. Applicant is a small entity; therefore, please charge Deposit Account number 26-0084 in the amount of \$210.00 for one month to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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